Supporting Information for

Molecular Imaging of Labile Iron(II) Pools in Living Cells with a Turn-on Fluorescent Probe

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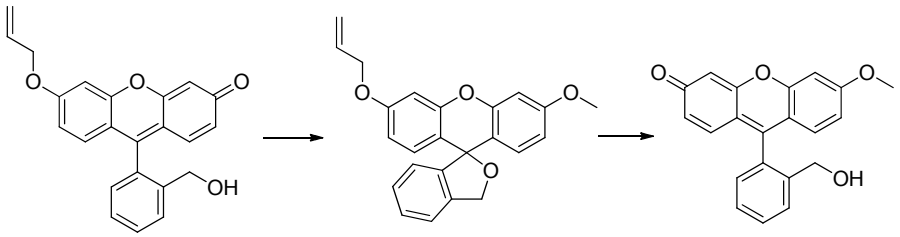
1. Synthesis

General. All solvents were of reagent grade. Acetonitrile, THF and dichloromethane (DCM) were dried by passing through activated alumina. All commercially purchased chemicals were used as received. Compound **4a** was synthesized according to literature procedures.[1] ^{1}H and ^{13}C NMR spectra were obtained from a Bruker AVB-400 NMR spectrometer at the College of Chemistry NMR facility at UC Berkeley. Signals were internally referenced to solvent residues. Low resolution mass spectral analyses were carried out using a LCMS (Agilent Technology 6130, Quadruploe LC/MS). High resolution mass spectral analyses (ESI-MS) were carried out at the College of Chemistry Mass Spectrometry Facility at UC Berkeley.



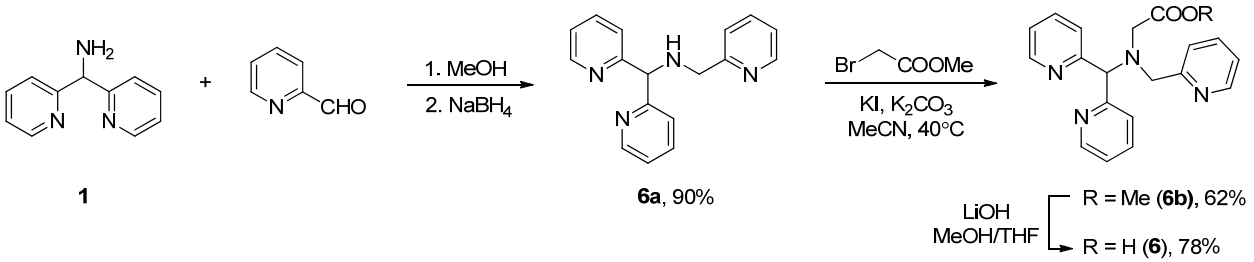
4a **4b**, quant. **4**, 87%

Synthesis of 4b. A mixture of **4a** (150 mg, 0.4 mmol), MeI (0.04 ml, 0.5 mmol) and Cs_2CO_3 (163 mg, 0.5 mmol) in 20 ml DMF was stirred at room temperature for overnight. The resulting mixture was diluted with ethyl acetate (200 ml) and washed with water (3 x 50 ml) and brine, and dried over Na_2SO_4. Solvent was removed to yield the product as an orange solid for use in the next step without further purification. Yield = 156 mg, quant. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 7.38 (d, *J* = 4.0 Hz, 2 H), 7.29–7.25 (m, 1 H), 6.92–6.76 (m, 3 H), 6.75 (dd, *J* = 2.4 Hz, 5.2 Hz, 2 H), 6.65 (dt, *J* = 2.4 Hz, 8.4 Hz, 2 H), 6.11–6.01 (m, 1 H), 5.44 (dd, *J* = 1.2 Hz, 17.2 Hz, 1 H), 5.42–5.30 (m, 3 H), 4.56 (dd, *J* = 1.2 Hz, 4.0 Hz, 2 H), 3.82 (s, 3 H). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$, 298 K) δ (ppm): 160.2, 159.2, 151.5, 151.4, 144.9, 139.1, 132.9, 129.8, 128.3, 128.0, 123.8, 120.6, 117.8, 117.3, 117.1, 111.6, 111.0, 101.3, 100.4, 83.5, 71.9, 68.9, 55.4. LRMS (ESI) calcd. for $C_{24}H_{21}O_4$ $[M+H]^+$ *m/z*: 373.1; found: 373.1.

Synthesis of 4. Compound **4b** (156 mg, 0.4 mmol) was dissolved in 20 ml THF. $Pd(PPh_3)_4$ (24 mg, 0.02 mmol) was added and the mixture was stirred under N_2 for 5 minutes, followed by addition of morpholine (0.08 ml, 0.9 mmol) and $NaBH_4$ (32 mg, 85 mmol). The mixture was stirred at room temperature under N_2 for overnight. The resulting mixture was diluted with 100 ml DCM, washed with water (2 x 30 ml) and brine, and dried over Na_2SO_4. Solvents were removed and the residue purified by silica column (hexanes/ethyl acetate = 50:50 → 25:75 → 0:100). Yield = 121 mg, 87%. ^{1}H NMR (400 MHz, d_6-acetone, 298 K) δ (ppm): 7.45 (d, *J* = 8.0 Hz, 1 H), 7.37 (t, *J* = 7.2 Hz, 1 H), 7.26 (t, *J* = 7.2 Hz, 1 H), 6.89 (d, *J* = 8.8 Hz, 1 H), 6.84–6.82 (m, 2 H), 6.73 (d, *J* = 2.8 Hz, 1 H), 6.67 (d, *J* = 2.8 Hz, 1 H), 6.65–6.57 (m, 2 H), 5.28 (s, 2 H), 3.82 (s, 3 H). ^{13}C{^{1}H} NMR (100.6 MHz, d_6-acetone, 298 K) δ (ppm): 161.2, 159.0, 152.1, 146.7,

[1] Ando, S.; Koide, K. *J. Am. Chem. Soc.* **2011**, *113*, 2556–2566.

140.0, 130.9, 130.8, 129.0, 128.8, 124.2, 121.8, 118.6, 117.7, 112.6, 111.7, 102.7, 100.9, 84.0, 72.7, 55.8. LRMS (ESI) calcd. for $C_{21}H_{17}O_4$ $[M+H]^+$ *m/z*: 333.1; found: 333.2.

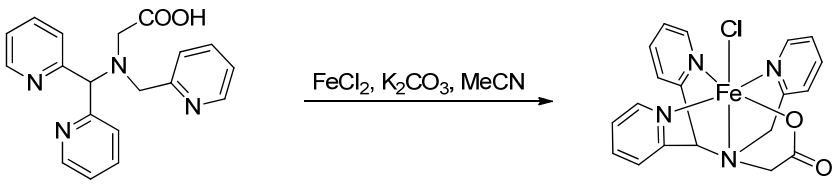


1 **6a**, 90%

Synthesis of 6a. A mixture of **1** (4.2 g, 22.6 mmol) and 2-pyridylcarboxyaldehyde (2.4 g, 22.4 mmol) in 50 ml MeOH was stirred at room temperature for 2 hours. The solution was cooled in an ice bath, $NaBH_4$ (2.1g, 56 mmol) was added in small portions. The mixture was slowly warmed to room temperature, and stirred for overnight. Solvent was removed and the residue re-dissolved in DCM, washed with water and brine and dried over Na_2SO_4. DCM was removed by a rotatory evaporator to give crude **6a** which was used in the next step without further purification. Yield = 5.6 g, 90%. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ (ppm): 8.59–8.55 (m, 3 H), 7.67–7.61 (m, 3 H), 7.49 (d, *J* = 8.1 Hz, 2 H), 7.35 (d, *J* = 7.8 Hz, 1 H), 7.19–7.15 (m, 3 H), 5.30 (s, 1 H), 4.02 (s, 2 H).

Synthesis of 6b. A mixture of **6a** (1.08 g, 3.9 mmol), methyl bromoacetate (0.6 g, 3.9 mmol), KI (65 mg, 0.4 mmol) and K_2CO_3 (0.54 g, 3.9 mmol) in 40 ml MeCN was heated at 40 °C for overnight. Insoluble material was removed by filtration and the filtrate was concentrated and purified by basic alumina column (100% ethyl acetate → ethyl acetate/MeOH = 95:5). Yield = 0.85 g, 62%. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 8.40–8.38 (m, 3 H), 7.63 (d, *J* = 7.6 Hz, 2 H), 7.54–7.50 (m, 4 H), 7.00–6.98 (m, 3 H), 5.45 (s, 1 H), 3.92 (s, 2 H), 3.47 (s, 3 H), 3.40 (s, 2 H). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$, 298 K) δ (ppm): 171.8, 159.9, 159.0, 149.1, 148.7, 136.4, 123.7, 123.0, 122.2, 122.1, 121.9, 72.8, 57.4, 51.4, 51.1.

Synthesis of 6. Compound **6a** (0.85 g, 2.43 mmol) was dissolved in a 1:1 mixture of MeOH and THF (10 ml each). LiOH (0.23 g, 9.6 mmol) was added and the mixture was stirred in room temperature for overnight. Solvents were removed by a rotatory evaporator and the residue re-dissolved in DCM. The DCM solution was partitioned in water and the pH of the aqueous phase was adjusted to ~7. The DCM phase was separated, dried over Na_2SO_4 and evaporated to dryness by a rotatory evaporator to give the product as an yellow oil. Yield = 0.63 g, 78%. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 11.23 (br s, 1 H), 8.40–8.36 (m, 3 H), 7.51–7.46 (m, 3 H), 7.26–7.24 (m, 3 H), 7.05–7.02 (m, 3 H), 5.36 (s, 1 H), 4.00 (s, 2 H), 3.41 (s, 2 H). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$, 298 K) δ (ppm): 174.3, 158.7, 158.2, 148.5, 148.0, 137.1, 136.8, 124.1, 122.8, 122.5, 122.3, 71.1, 58.1, 54.5.



6 **6**-FeCl

Synthesis of 6-FeCl. Under an N_2 atmosphere, a mixture of **6** (0.33 g, 0.1 mmol) and K_2CO_3 (0.14 g, 0.1 mmol) was stirred in MeCN (15 ml) for 30 min at room temperature. A slurry of $FeCl_2$ (0.13 g, 0.1 mmol) in MeCN (5 ml) was added and the mixture was stirred for overnight. Insoluble material was removed by filtration and the filtrate was slowly evaporated to give the neutral Fe(II) complex as red crystals. ^{1}H NMR (400 MHz, CD_3CN, 298 K) δ (ppm): 97.3, 88.2, 59.0, 57.4, 54.3, 51.8, 49.4, 40.7, 40.1, 24.4, 24.2, 23.5, 22.0, -33.3. ESI-MS (+ve): 425.0 $[M+H]^+$ (100%), 389.0 $[M-Cl]^+$ (56%), EI-HRMS: 424.0386; calc. for $C_{19}H_{17}N_4O_2{}^{35}Cl^{56}Fe$: 424.0384. UV-Vis (MeCN): λ = 404 nm (ε = 390 $cm^{-1}M^{-1}$), 496 nm (ε = 360 $cm^{-1}M^{-1}$). FT-IR (solid): 1600 cm^{-1}, 1360 cm^{-1}.

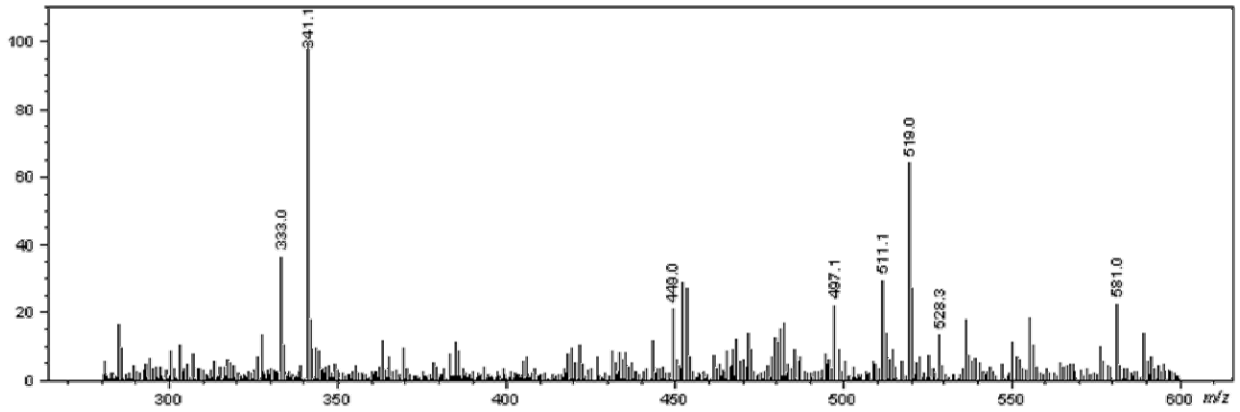


Fig. S1. ESI-MS (+ve) spectrum of reaction mixture of IP1 and 20 eq. Fe^{2+} after 1 hour. Release of hydroxymethyl fluorescein **3** is indicated by the presence of the peak at *m/z* = 333.0. The peak at *m/z* = 449.0 is assigned to the iron complex with the oxidized ligand $[Fe(L\text{-}COO)]^{+}$.

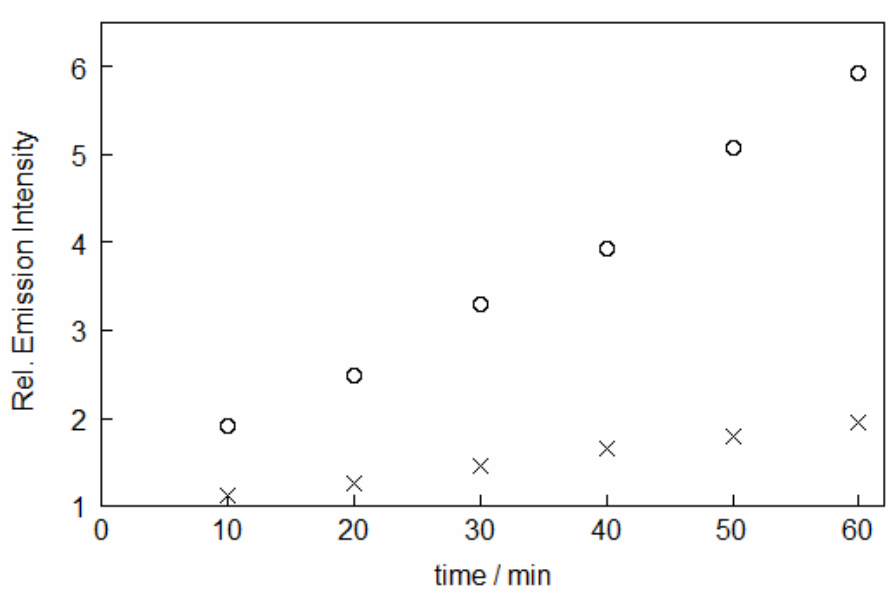


Fig. S2. Relative emission intensity at 505 nm of IP1 and 20 eq. Fe^{2+} under aerobic (o) and anaerobic (x) conditions over time. λ_{ex} = 470 nm.

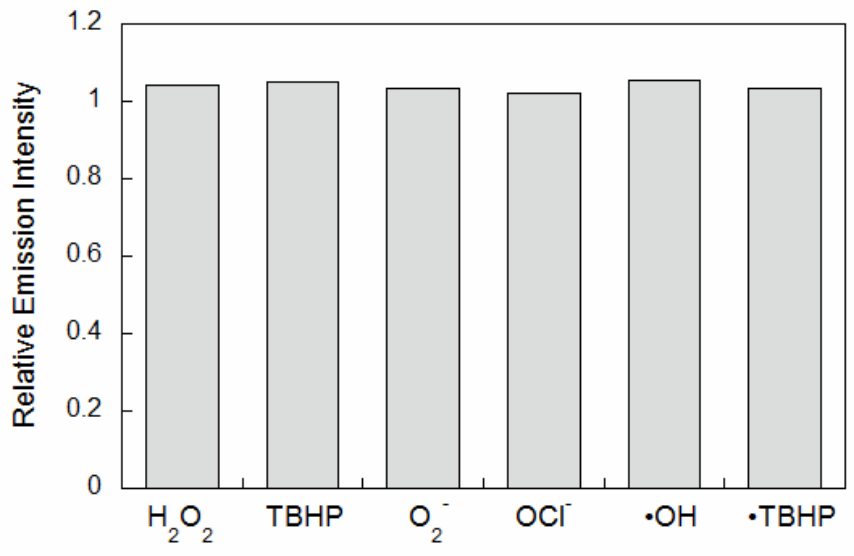


Fig. S3. Relative fluorescence intensity at 505 nm of 1 μM IP1 after a 1-hour reaction with 100 equivalents of various ROS.

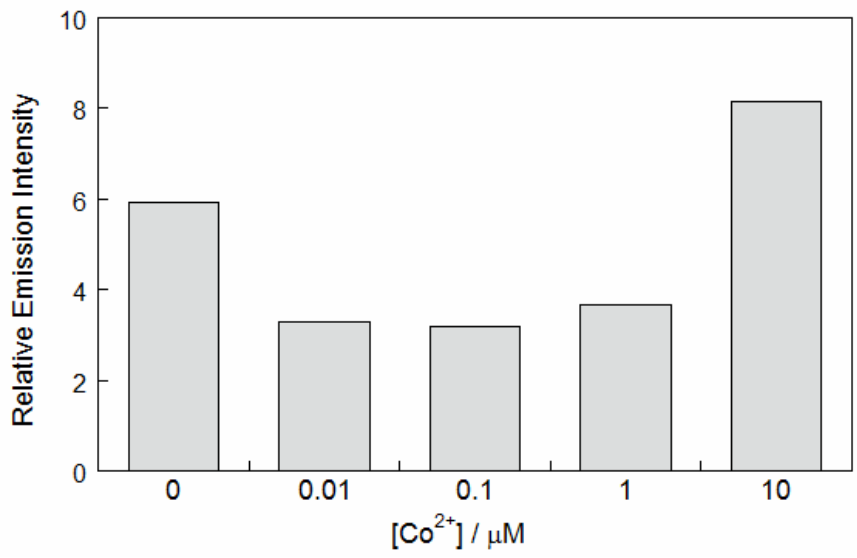


Fig. S4. Relative fluorescence intensity at 505 nm of 1 μM IP1 after a 1-hour reaction with 10 μM Fe^{2+} in the presence of various concentrations of Co^{2+}.

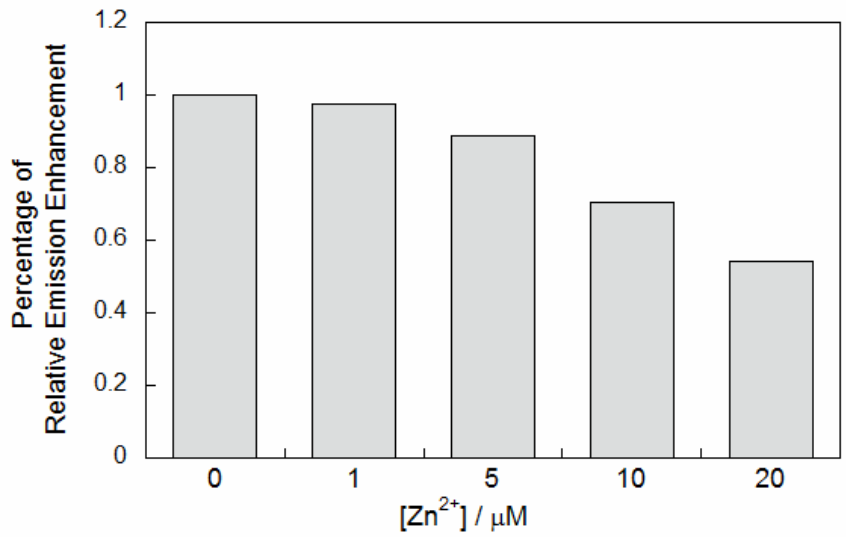


Fig. S5. Percentage change in relative fluorescence intensity at 505 nm of 1 μM IP1 after a 1-hour reaction with 20 μM Fe^{2+} in the presence of various concentrations of Zn^{2+}.

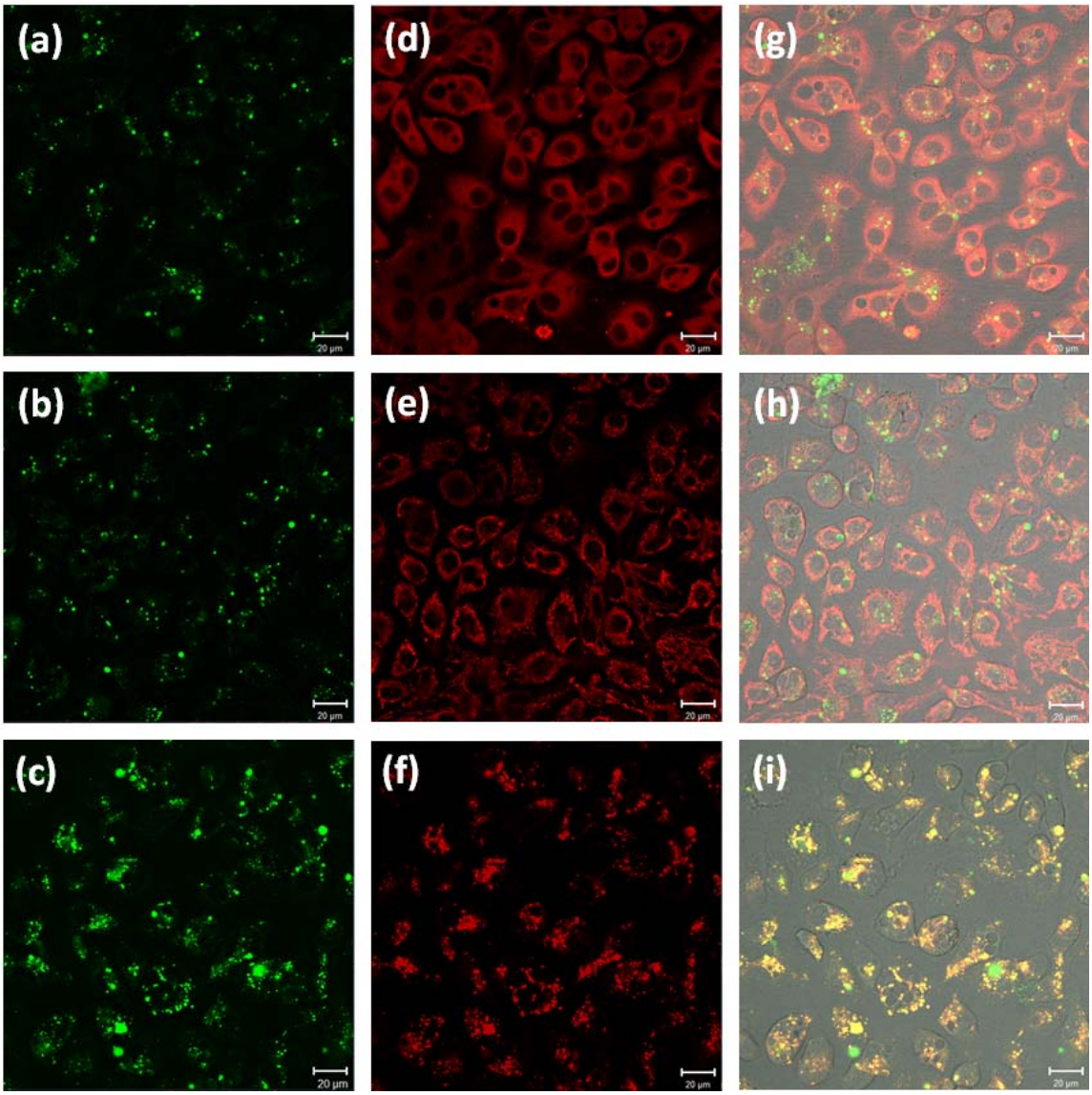


Fig. S6. Confocal microscopy images of HepG2/C3A cells loaded with (a-c) 10 μM IP1; (d) 100 nM ER-Tracker Red; (e) 100 nM Mitotracker Red; (f) 100 nM Lysotracker Red; (g-i) Overlays of bright field images, IP1, and the various trackers.

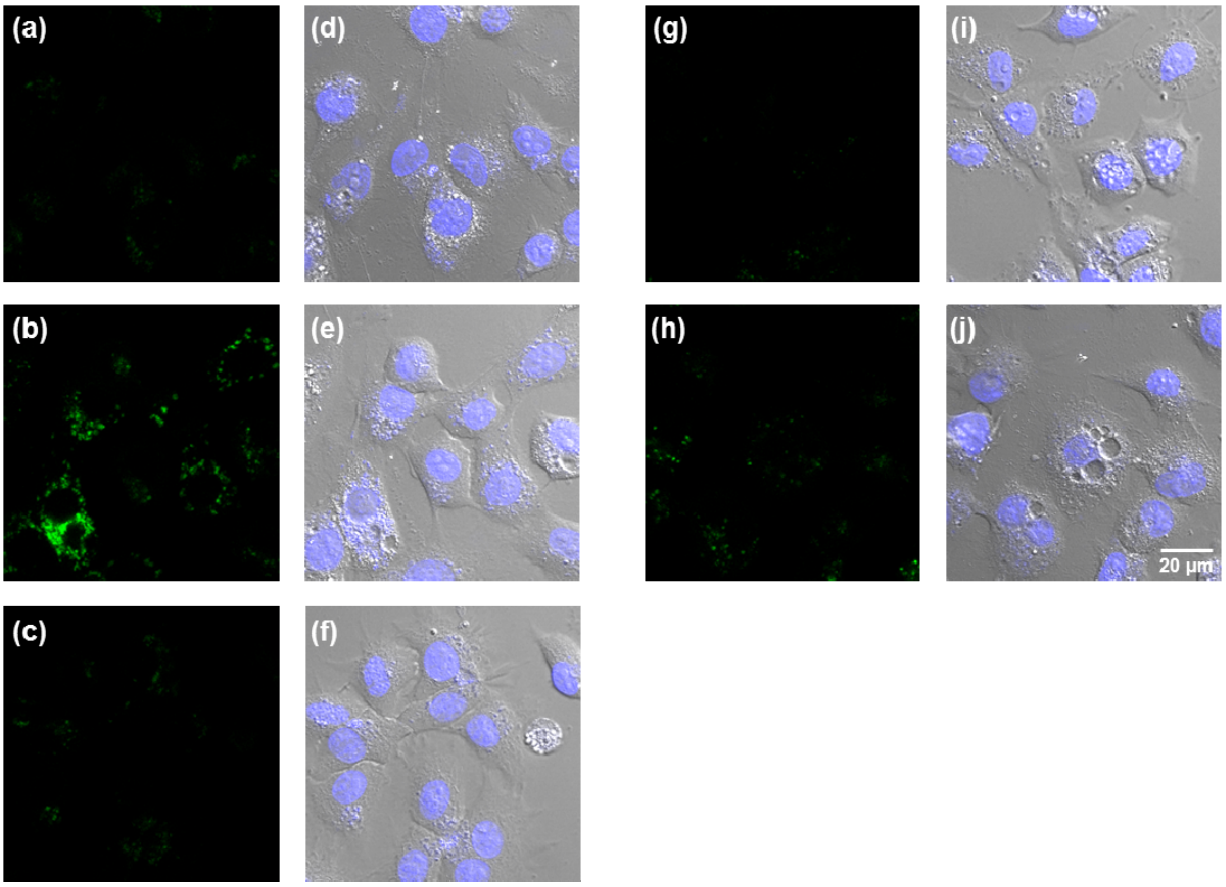


Fig. S7. Confocal microscopy images of HepG2/C3A cells loaded with 10 µM CP1: (a) control; (b) with 100 µM $CoCl_2$ for 20 hours; (c) with 100 µM FAS for 20 hours; (g) with 100 µM FAS for 20 hours and 0.5 µM hepcidin for 19 hours; (h) with 1 mM ascorbic acid for 40 minutes. Overlays of bright field images and Hoechst stain (d–f, I and j) of respective images are shown on the right.

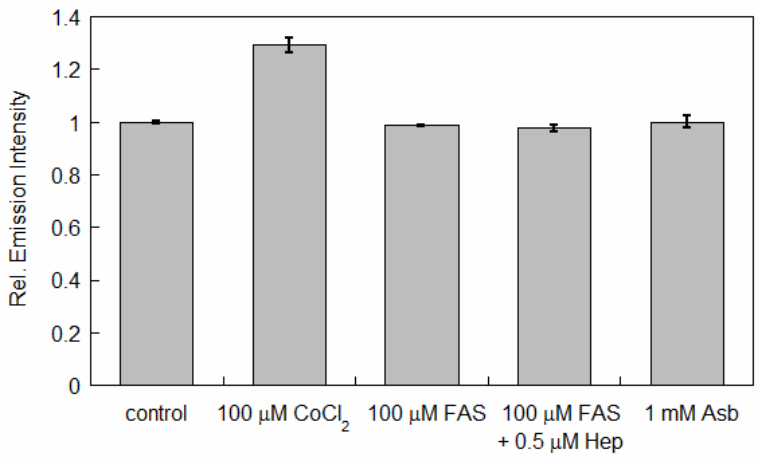


Fig. S8. Relative emission intensity of confocal microscopy images of HepG2/C3A cells stained with 10 µM CP1. Error bars are ±SD (n = 3).

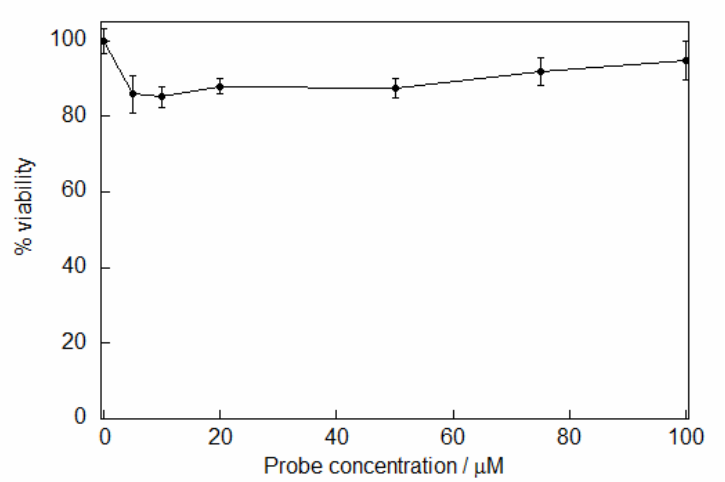


Fig. S9. Percentage viability of HepG2/C3A cells at different IP1-AM concentrations for 2 hours by WST-1 assay. Error bars are ±SD (n = 3).

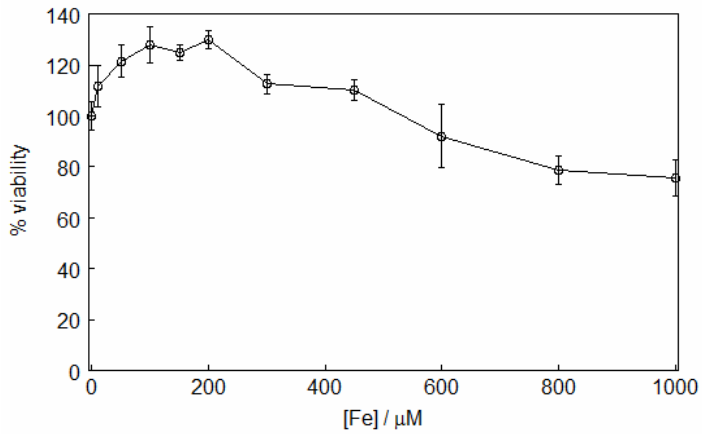


Fig. S10. Percentage viability of HepG2/C3A cells at different FAS concentrations for 20 hours by WST-1 assay. Error bars are ±SD (n = 3).

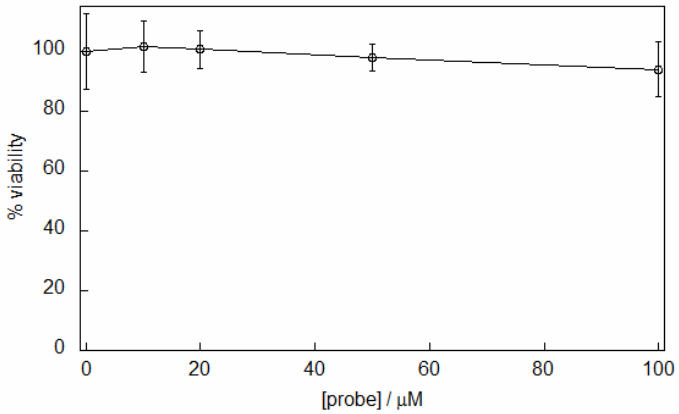


Fig. S11. Percentage viability of HepG2/C3A cells enriched with 100 µM FAS at different IP1-AM concentrations for 2 hours by WST-1 assay. Error bars are ±SD (n = 3).

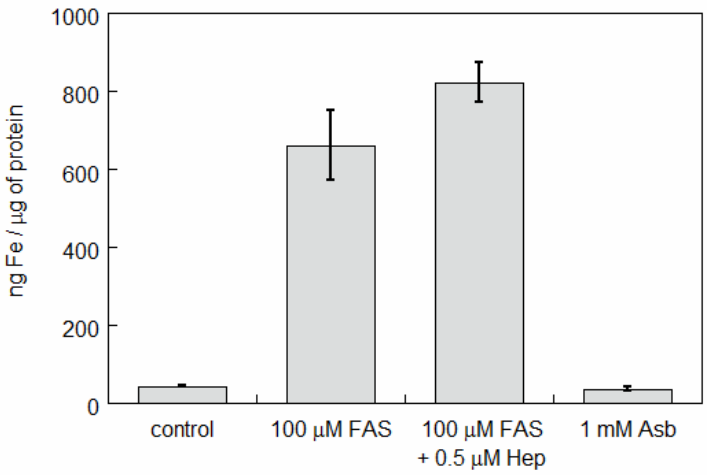


Fig. S12. Total iron content in cell lysates of HepG2/C3A cells as determined by ICP-OES. Protein content was determined by BCA assay. Error bars are ±SD (n = 3).

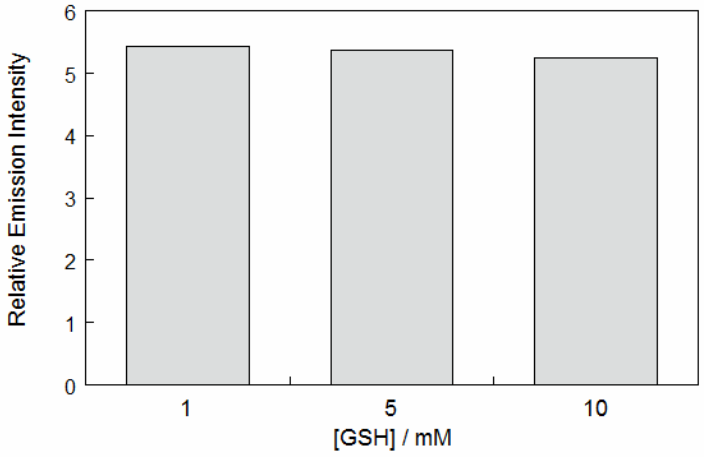


Fig. S13. Relative fluorescence intensity at 505 nm of 1 µM IP1 after a 1-hour reaction with 20 µM Fe^{2+} in the presence of various concentrations of GSH.

Table S1. Crystal data and structure refinement for [Fe**6**Cl]

Empirical formula	C21 H20 Cl Fe N5 O2	
Formula weight	465.72	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/n	
Unit cell dimension	a = 8.6726(6) Å	α = 90°
	b = 12.8597(8) Å	β = 92.648(4)°
	c = 19.4545(12) Å	γ = 90°
Volume	2167.4(2) $Å^3$	
Z	4	
Density (calculated)	1.427 Mg/m^3	
Absorption coefficient	0.846 mm^{-1}	
F(000)	960	
Crystal size	8 x 5 x 2 mm^3	
θ rage for data collection	1.90 to 25.47°	
Index ranges	-10<=h<=7, -15<=k<=15, -23<=l<=23	
Reflections collected	21054	
Independent reflections	3991 [R(int) = 0.0603]	
Completeness to θ = 25.00°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.2824 and 0.0564	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3991 / 257 / 257	
Goodness-of-fit[a] on F^2	1.033	
Final R indices[b] [I>2 sigma(I)]	R_1 = 0.0715, wR_2 = 0.1842	
R indices[b] (all data)	R_1 = 0.1044, wR_2 = 0.2031	

[a]GooF = $[\sum[w[(F_o^2 - F_c^2)^2]/(n-p)]^{1/2}$

[b]$R_1 = \sum||F_o| - |F_c|| / \sum|F_o|$, $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2 / \sum[w(F_o^2)^2]]^{1/2}$